

NOTE UPON THE EXTRACTION OF ACIDS FROM CULTURES.

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In testing the products of the fermentation of dextrose by a film yeast, succinic acid was obtained as the only fixed acid, but during its separation, several observations were made which may be of interest.

The fermented fluids are usually evaporated in neutral or alkaline solution, generally in the presence of an excess of calcium carbonate and when the liquid is sufficiently reduced, it is cooled and the lime salts decomposed with sulphuric acid, after which the calcium sulphate is removed by filtration and the volatile acids removed in a current of steam.

The residual fixed acids are then evaporated until of sufficient consistency to be extracted with ether. There may be, and usually is, a quantity of sugar in the liquid which prevents the evaporation being pushed to a limit, say, of 20 c.c. or which does not give a tractable dry powder when mixed with sand. In such cases one has to work with a volume of liquid measuring possibly 100 c.c. With this volume, the extraction is slower than is generally considered. For example, Schoorl (through Journ. Soc. Chem. Ind., xix., 1900, 567), when working with pure acids, found that succinic, lactic and oxalic acids were extracted from 20 c.c. of liquid in 8 hours. The time may be longer in the presence of much sugar and nitrogenous substances, but this has not been investigated. Schoorl states that the time of extraction depends upon the partition equivalent, which is the solubility of the acid in water.

An acid such as succinic with a low coefficient solubility of the acid in ether. takes a short time, and one with a high coefficient such as tartaric acid takes a long time. He gives the coefficients as succinic, 4; oxalic, 8; lactic, infinity; malic, 24; citric, 59; and tartaric, 330. In extracting unknown acids as, for example, in a bacterial culture, one naturally continues the extractions until little or no residue is obtained upon evaporating the ether from a three hours' extraction.

More recently Pinnow (Jour. Soc. Chem. Ind., xxxv., 1916, 197) determined the coefficients of distribution between certain organic acids in water and in ether and compared the speed of their extraction by ether in a Partiel-Rose extraction apparatus. He found that the extraction practically followed the formula for monomolecular reactions and, within certain limits, was inversely proportional to the volume of liquid extracted and to the distribution coefficient. His coefficients at 27° are for succinic acid, 7.58; lactic, 11.28; oxalic, 13.9;

malic, 70.9; and citric, 153. The time in minutes required for a 99 % extraction in a Parthiel-Rose apparatus holding 30 c.c. is obtained by dividing the Distribution Equivalent by 0.04.

From this one gathers that lactic acid is extracted in 4½ hours in a Parthiel-Rose apparatus holding 30 c.c., while in a Schoorl apparatus it took 8 hours dealing with 20 c.c. The kind of apparatus is therefore of some moment. The Schoorl apparatus can easily be obtained and is the one I have used. A test with it upon 100 c.c. of liquid containing 0.25 grams of succinic acid showed that 79 % was extracted in 7 hours and 99 % in 12 hours. The presence of 5 % of dextrose made no difference in the speed of extraction.

The fixed acids from the film yeast were contained in a fairly strong solution of sugar with ammonium and other salts and measured about 100 c.c. After certain periods of extraction the bulk of the ether was distilled off, the remainder air-evaporated and the residue titrated with tenth-normal soda.

Period of extraction	Acid extracted as c.c. of tenth normal.		
7 hours	48.4	or 53 %	
19 hours	23.3	71.7	78 %
31 hours	9.3	81.0	86 %
43 hours	5.5	86.5	94 %
55 hours	5.3	91.8	100 %

In another test the acids had been extracted and the calcium salts, insoluble in 70 % alcohol, were treated with sulphuric acid and percolated with ether. The volume measured 50 c.c.

Period of extraction	Acid extracted as c.c. of tenth normal.		
7 hours	81.83	or 61 %	
19 hours	29.95	111.78	83 %
31 hours	15.05	126.83	95 %
43 hours	6.93	133.76	100 %

Curves prepared from these figures showed that they were of the nature of monomolecular reactions, while an extended examination of the portions showed that succinic acid was the only acid present. The examination was extended because succinic acid is not so definite in its behaviour as one is led to suppose. The slight solubility of the calcium and of the silver salts causes it to appear in places where other acids are expected. For example, while a pure salt of succinic acid was completely precipitated by silver nitrate and contained the theoretical amount of silver, the neutral salt of the acid obtained from the yeast culture was not completely precipitated, for the quantity was lower than was expected from the neutralisation numbers and the filtrate contained a considerable amount of the acid. So much so that it almost appeared as if there were more than one kind of succinic acid. The melting point was normal while the silver content of the silver salt was low, 62.4 and 62.6, as against the theoretical 65.07 %. There was always a small quantity of a syrupy substance associated with the acid. It was too small in amount to determine its nature, but it influenced the crystallisation of the pure acid. It is possible that it may have had something to do with the solubility of the silver salt.

An attempt was made to determine the nature of this syrupy acid. The mother-liquor, after the 55 hours' extraction (see above) was treated with an

excess of baryta, and evaporated to small volume, with the idea of hydrolysing any acid esters. The liquid was treated with sulphuric acid and extracted for 12 hours with ether. The residual acids were neutralised with 9.15 c.c. of N/10 baryta water and were treated with sufficient alcohol to make a 75 % solution. The precipitated barium succinate was filtered off and dried at 130° when it gave 0.0416 gram which contained 54.76 % Ba. Barium succinate contains 54.16 %. The filtrate, when evaporated and dried at 130°, gave 0.1038 gram containing 33.33 % Ba. The barium succinate corresponded to 3.29 c.c. N/10, which left 5.86 c.c. for the acid in the alcohol-soluble salt. Deducting the Ba. and allowing for the H₂ equivalent, the acid was calculated as weighing 0.0697 gram. This gave a neutralisation equivalent of 119 and, as we have seen, the Ba. content was 33.33 %. Isovaleric or levulinic acid is indicated, but the quantity worked upon was very small for absolutely definite identification.

As a matter of fact, the substance was probably lactic acid, for in working through another culture, the succinic acid was precipitated as the barium salt in 75 % alcohol and the filtrate, after evaporation, acidification and extraction with ether, yielded a syrupy residue which, when boiled with zinc oxide, furnished crystals of zinc lactate.

One has to be very careful in deducing the nature of the acid from small quantities of material as, even with comparatively large quantities, one may be led into error. For example, a culture of the same yeast was made with dextrose, ammonium nitrate, and sodium phosphate in the presence of chalk. In working up the products, the ethereal solution was evaporated in the air and then distilled in a current of steam. On concentrating the solution on the water bath a disengagement of nitric oxide occurred. The nitric acid had formed nitrous ether during a part of the process, and this had not been removed by the steam and had decomposed on warming in the open. The residual acids were neutralised with baryta water in the presence of phenolphthalein to a permanent pink colour, when a precipitate of barium phosphate and oxalate was thrown down. The filtrate on concentration threw down successive crops of a crystalline precipitate containing 47.8 % and 48.9 % of Barium. A portion of the precipitate acidified and extracted with ether yielded crystals of succinic acid melting at 183°. The mother liquor now required a considerable quantity of baryta water to give it a permanent pink tint. Thus the original addition of baryta to the first solution sufficient to give a permanent pink colour was not sufficient to produce normal barium salts, for if so the amount of Barium in the precipitated succinate would have been nearer the theoretical 54.16 % and the further addition of baryta to the mother liquor would have been unnecessary.

Summary.—The extraction of the fixed acids from bacterial or from yeast cultures is, as has been shown by other writers, of the nature of a monomolecular reaction and should be continued until no more acid is extracted.

The preparation of salts, such as those of barium, by neutralising the extracted acids until a pink colour is obtained in the presence of phenolphthalein, may be faulty as the reaction is much slower than is generally supposed.